MRI Monitoring of Stem Cell Therapy

G.A. Walter, UF, Physiology and Functional Genomics; McKnight Brain Institute; Powell Gene Therapy Center; UF Cancer Center; and the NHMFL
K. Vandenborne, UF, Physical Therapy; McKnight Brain Institute; and the NHMFL
B.J. Byrne, UF, Pediatrics and Molecular Genetics and Microbiology; and Powell Gene Therapy Center
K.S. Cahill, UF, Molecular Genetics and Microbiology; and Powell Gene Therapy Center
T.N. Frimel, UF, Physical Therapy
G.S. Gaidosh, UF, Physiology and Functional Genomics

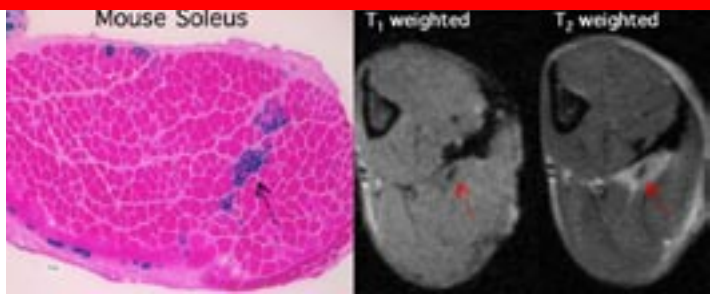

Figure 1. (Left) Histological verification of muscle stem cell integration into the mouse soleus following muscle damage by X-gal staining of the LacZ expressing cells (blue). (Right) Labeled cells result changes in MR image contrast on both T1 and T2 weighted sequences. Note the hyper intense regions within the mouse soleus on T2 weighted images correspond to muscle damage prior to stem cell delivery.

With new potential therapies on the horizon, there is an urgent need for noninvasive imaging modalities to study muscle function. Gene and/or stem cell transfer, two therapeutic strategies that may have tremendous therapeutic potential, currently rely heavily on invasive techniques. This is problematic when attempting to evaluate these therapies in the clinical setting involving patients with extensive muscle damage or during muscle senescence. Therefore, it is imperative to develop noninvasive techniques capable of providing high-resolution images of muscle at the cellular level. Current work performed in the AMRIS facility focuses on the development of magnetic resonance spectroscopy (MRS) and imaging (MRI) techniques that allow for the noninvasive monitoring of gene transfer efficacy and stem cell delivery methods. Widespread gene expression can be achieved in adult cardiac and skeletal muscle using either recombinant adeno or adeno-associated viruses. We have previously found that MRI/MRS methods can be used to monitor the expression of a MR marker gene (arginine kinase)[1] and therapeutic genes for the muscular dystrophies and cardiomyopathies.[2] On the other hand, cell based therapies may represent a greater challenge for noninvasive monitoring due to the wide variability in stem cell incorporation.

Recent interest has focused on the therapeutic use of stem cell transplants for diseases characterized by irreplaceable cell loss. The identification of novel populations of adult muscle stem cells capable of multi-potent differentiation and enhanced muscle regeneration has fueled new interest in the clinical promise of cell-based therapies for muscular dystrophy and muscle senescence.[3,4] It is anticipated that such a population of adult cells can be isolated, modified to express therapeutic genes, and then reintroduced systemically. *In vivo* cell migration to damaged areas occurs by the local expression of endogenous chemotatic and mitogenic factors. We are addressing whether cell homing can be enhanced by the targeted expression of key chemotatic genes by recombinant viral techniques. In Duchenne's muscular dystrophy, for example, a mutation in the gene encoding the membrane protein dystrophin ultimately leads to the exhaustion of resident muscle progenitor cells in an attempt to replace damaged and dysfunctional muscle fibers. Once unable to further regenerate damaged fibers, the muscle is quickly infiltrated by connective tissue and fat.[5] Therapeutic approaches have been designed to replace dysfunctional muscle fibers and complement dystrophin deficiency through the delivery of normal or genetically modified stem cells

capable of muscle fiber regeneration.[6] A second example is senescent and damaged tissue in which resident stem cells may be exhausted or non-functional. In this experimental paradigm MR is used to determine whether the recruitment of endogenous and/or mobilized stem cells will increase the rate of muscle regeneration and improve muscle function.

Common techniques to monitor muscle stem cell transplants typically rely on *ex vivo* genetic modification to allow expression of reporter genes.[7] The statement of specific reporter genes allows for graft identification during post-mortem analysis. Using these conventional techniques, however, even simple and practical questions are difficult and labor intensive to answer. For example, to determine the distribution of the graft, the entire organ must be harvested and sectioned, followed by identification of individual cells by conventional microscopy. More complex questions, such as cell homing, identification of migration events, and engraftment rates, may be impossible to accurately and quantitatively address using conventional microscopy. Novel techniques that allow non-invasive, continuous imaging of stem cell transplants have recently been proposed and evaluated in a limited number of cell delivery models.[8,9] MRI imaging has the ability to provide extremely sensitive, high-resolution images of magnetically labeled cells. As such, the application of MR imaging to stem cell investigations is of great importance to enhance the development of stem cell therapies.

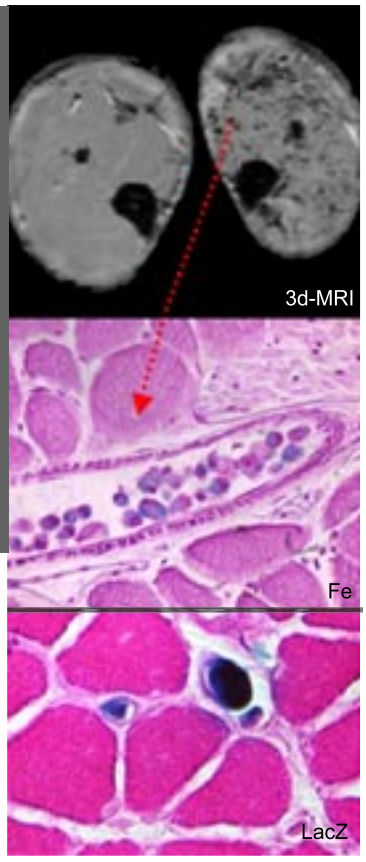

Figure 2.
Detection of labeled stem cells following arterial delivery to mouse hindlimb muscles. One entire hindlimb was occluded during cell injection (top; right) whereas the contra lateral limb's blood flow was unaltered (top; left). Labeled cells appeared as dark regions on 3d-MRI images (top; right). Iron containing (middle) and LacZ expressing stem cells (bottom) were found within the vessels of the limb with normal blood flow

We are currently evaluating the application of magnetically labeled stem cells for monitoring therapeutic stem cell transplants in murine dystrophies[13] and senescent muscle (Figure 1). Multipotent, muscle derived stem cells are labeled by incubation with ferumoxide:polycation complexes resulting in endosomal accumulation of supraparamagnetic iron-oxide. The presence of a small number of labeled cells causes large changes in MR contrast (decreased T_2&T_2*), allowing for three dimensional, non-invasive detection. Relaxativity measurements on cell phantoms demonstrate that MR imaging can be used to detect a single labeled cell. Furthermore, therapeutic cellular grafts transplanted into dystrophic muscle and normal murine muscles can be imaged sequentially, showing a strong spatial correlation between *in vivo* and *in vitro* indices of cell incorporation. Additional studies reveal that MR imaging can be implemented to track the migration of a small number of labeled cells following arterial delivery (Figure 2).

Targeting stem cells to the ischemic and damaged myocardium is of the highest priority. Cardiac dysfunction, resulting from various insults to the myocardium, can ultimately lead to the development of heart failure. The heart is at great risk of irreversible cell loss due to the limited regenerative capacity of adult cardiac tissue and the lack of a resident cardiac progenitor cell equivalent to the skeletal muscle satellite cells. The transplantation of myogenic cells into the myocardium has been investigated as a novel mechanism to repair damaged and dysfunctional myocardium.[10] Skeletal myoblasts are a committed progenitor cell population found in close proximity to the mature skeletal myocytes.[11] This cell population can be easily isolated from an adult muscle biopsy and propagated in tissue culture. Numerous reports have documented the ability of skeletal myoblasts to survive in healthy as well as damaged myocardium and ultimately improve systolic and diastolic function.[12] Again progress in the development and implementation of stem cell transplants as clinical therapies has been delayed by limitations in post-mortem analyses. MR cardiac imaging has the dual advantage of being able to provide high-resolution images that not only offer structural information, but can be further utilized to provide indices of global and regional cardiac function. We have demonstrated that small numbers of magnetically labeled myoblast grafts can be detected in rodent myocardium following transplantation (Figure 3). The non-invasive analysis of myoblast grafts will be important in clinical studies to readily determine the relationship of the graft to cardiac function.

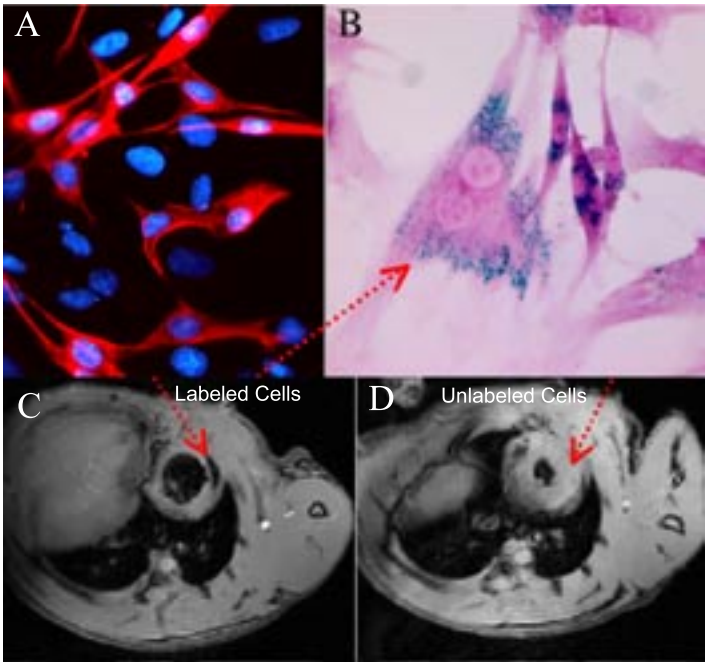

Figure 3. (A) Desmin expression (red) by primary rat myoblast cultures after 5 days of culture. (B) Prussian blue stain of undifferentiated following ferumoxide-PLL labeling. Short axis (C&D) MRIs of labeled (C) and unlabeled (D) myoblast transplants in viable rat myocardium 24hrs post-injection.

We conclude that MR monitoring of contrast agent loaded cells is particularly well suited for investigations involving the vascular delivery of stem cells and other delivery strategies that rely on stem cell homing to the site of injury. This MR technique utilizes FDA approved contrast agents, is widely accessible, and may permit continuous, non-invasive readout of cell transplants in cardiac and skeletal muscle.

1 Walter, G.; Barton, E.R. and Sweeney, H.L., "Noninvasive measurement of gene expression in skeletal muscle," *Proc. Natl. Acad. Sci. U.S.A,* **97**, 10, 5151-5155 (2000).

2 Fraites, T.J., Jr.; Schleissing, M.R.; Shanely, R.A.; Walter, G.A.; Cloutier, D.A.; Zolotukhin, I.; Pauly. D.F.; Raben N.; Plotz, P.H.; Powers, S.K.; Kessler, P.D. and Byrne, B.J., "Correction of the enzymatic and functional deficits in a model of Pompe disease using adeno-associated virus vectors," *Mol. Ther.*, 5, (5 Pt 1), 571-578 (2002).

3 Jankowski, R.J.; Deasy, B.M.; Cao, B.; Gates, C. and Huard, J., "The role of CD34 expression and cellular fusion in the regeneration capacity of myogenic progenitor cells." *J. Cell. Sci.*, 115, (Pt 22), 4361-4374 (2002).

4 Sampaolesi, M.; Torrente, Y.; Innocenzi, A.; Tonlorenzi, R.; D'Antona, G.; Pellegrino, M.A.; Barresi, R.; Bresolin, N.; De Angelis, M.G.; Campbell, K.P.; Bottinelli, R. and Cossu, G. "Cell therapy of alpha-sarcoglycan null dystrophic mice through intra-arterial delivery of mesoangioblasts," *Science;* 301, 5632, 487-492 (2003).

5 Emery, A.E., "The muscular dystrophies," *Lancet*, 359, 9307, 687-695 (2002).

6 Cossu, G. and Mavilio, F, "Myogenic stem cells for the therapy of primary myopathies: wishful thinking or therapeutic perspective?" *J. Clin. Invest.*, **105**, 12, 1669-1674 (2000).

7 Toma, C.; Pittenger, M.F.; Cahill, K.S.; Byrne, B.J. and Kessler P.D., "Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart," *Circulation*, **105**, 93-98 (2002).

8 Allport, J.R. and Weissleder, R., *In vivo* imaging of gene and cell therapies. *Exp. Hematol.*, 29, 11, 1237-1246 (2001).

9 Weissleder, R., "Molecular imaging: exploring the next frontier," *Radiology*, **212**, 3, 609-614 (1999).

10 Nadal-Ginard, B.; Kajstura, J.; Leri A. and Anversa P., "Myocyte death, growth, and regeneration in cardiac hypertrophy and failure," *Circ. Res.*, **92**, 139-150 (2003).

11 Hawke, T.J. and Garry D.J., "Myogenic satellite cells: physiology to molecular biology," *J. Appl. Physiol.,* **91**, 534-551 (2001).

12 Kessler, P.D. and Byrne, B.J., "Myoblast cell grafting into heart muscle: cellular biology and potential applications," *Annu. Rev. Physiol.*, **61**, 219-242 (1999).

13 Walter, G.; Cahill, K.C.; Feng, H.; Douglas, T.; Huard, J.; Sweeney, H.L. and Bulte, J.W.M., "Noninvasive Monitoring of Myoblast Transfer for the Treatment of Muscular Dystrophies" (In press *Magnetic Resonance in Medicine*).

S_C COIL INSERT from page 18.

7 J. Schwartz; J.S. Brooks; T. Cross; K. Marken; M. Meinesz; H. Miao; U.P. Trociewitz and H.W. Weijers, "The creation of a 25 T magnetic field in a 38 mm bore solenoid using a superconducting insert magnet," *Nature*, (submitted December 2003).

8 H.W. Weijers; Q.Y. Hu; Y. Viouchkov; E. Celik; Y.S. Hascicek; K. Marken; J. Parrell and J. Schwartz, 2000, Development and testing of a 3 T Bi-2212 insert magnet, *Advances in Cryogenic Engineering*, **45**, 769-778 (2000).

9 K.R. Marken; H. Miao; M. Meinesz; B. Czabaj and S. Hong, BSCCO-2212 Conductor Development at Oxford Superconducting Technology, *IEEE Trans. Appl. Supercond.*, **13**, 3335-3338 (2003).

10 A.L. Mbaruku; K.R. Marken; M. Meinesz; H. Miao; P.V.P.S.S. Sastry and J. Schwartz, Effect of processing defects on stress-strain-I_c for AgMg sheathed Bi-2212 tapes, *IEEE Trans. Appl. Supercond.,* **13**, 3522-3525 (2003).

11 P.H. Kes; J. Aarts; V.M. Vinokur and C.J. van Beek, "Dissipation in highly anisotropic superconductors," *Phys Rev. Letters*, **4**, 1063-1066 (1990).

12 H.W. Weijers; J. Schwartz; B. ten Haken and H.H.J. ten Kate, "Effects of conductor anisotropy on the design of Bi-Sr-Ca-Cu-O sections of 25 T solenoids," *Supercond. Sci. Technol.*, **16,** 1-10 (2003).

13 I.H. Mutlu; E. Celik and Y.S. Hascicek, "High temperature insulation coating and their electrical properties for HTS/LTS conductors," *Physica C*, **370**, 2, 113-124 (2002).

14 M. Okada; K. Tanaka; T. Wakuda; K. Ohata; J. Sato; T. Kiyoshi; H. Kitaguchi; H. Kumakura; K. Togano and H. Wada, "Bi-2212/Ag multifilamentary tapes, wires and coils for high magnetic field applications," *Adv. in Superconductivity XI*, 851-854 (1999).